Journal of Photochemistry & Photobiology, B: Biology 176 (2017) 62–68

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

The interaction between the light source dose and caspase-dependent and -independent apoptosis in human SK-MEL-3 skin cancer cells following photodynamic therapy with zinc phthalocyanine: A comparative study

Mohammad Amin Doustvandi[a], Fateme Mohammadnejad[a], Behzad Mansoori[a,b], Ali Mohammadi[a], Farzaneh Navaeipour[c], Behzad Baradaran[a], Habib Tajalli[d,*]

[a] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Faculty of Physics, Iran University of Science and Technology, Tehran, Iran
[d] Research Institute for Applied Physics and Astronomy, University of Tabriz, Tabriz, Iran

ARTICLE INFO

Keywords:
Photodynamic therapy
Zinc phthalocyanine
SK-MEL-3
Caspase-dependent apoptosis
Caspase-independent apoptosis
Laser diode

ABSTRACT

The aim of this study is to determine the behavior of relative expression of Bcl-2, caspase-8, caspase-9, and caspase-3 genes of/in SK-MEL-3 cancer cells and explore molecular mechanisms responsible for the apoptosis response during an in vitro photodynamic therapy (PDT) with Zinc Phthalocyanine (ZnPc) using different doses of the light source. In this study, firstly the cytotoxic effects of ZnPc-PDT on SK-MEL-3 cells were evaluated. By irradiating the laser, ZnPc induced a significant amount of apoptosis on SK-MEL-3 cells in three IC_{50}s including 0.064 ± 0.01, 0.043 ± 0.01, and 0.036 ± 0.01 μg/mL at the doses of 8, 16, and 24 J/cm^2, respectively. Moreover, flow cytometry and QRT-PCR experiments were done. The high percentage of apoptotic cells was seen in the early apoptosis stage. The expression of Bcl-2 and caspase-8 genes at all doses of laser experienced an obvious reduction in comparison to the control group. On the other hand, although the expression of caspase-9 and caspase-3 genes remains almost constant at 8 J/cm^2, but they faced an increment at 16 and 24 J/cm^2 doses. These data reveal caspase-dependent apoptosis in high and caspase-independent apoptosis in low doses of laser. Based on the results of present work, it can be suggested that the dose of the light source is a key factor in induction of caspase-dependent and caspase-independent apoptosis pathways following PDT.

1. Introduction

Photodynamic therapy (PDT) is a new approach for cancer treatment with the minimum invasive effect on non-cancer tissues, which functions by exciting a non-toxic light-sensitive compound (photosensitizer) using a laser light [1,2]. This therapy has been used to treat cancers including head and neck [3], esophageal [4], bladder [5], liver [6], lung [7], breast [8], brain tumors [9], Kaposi's sarcoma, oral cavity [10,11], skin [12], prostate [13], the cervical [14], and gastrointestinal cancers [15] with promising results in comparison to other cancer therapies.

The main problems of conventional cancer therapies including chemotherapy, radiation therapy, hormone therapy, and surgery are high toxicity, serious side effects, and damage to the healthy tissues [16]. Meanwhile, in PDT, photosensitizer delivery to the target tissues and focused light source precisely on them, can limit cell damage in the treated area and consequently reduce the damage inflicted to normal tissue [17].

PDT itself consists of three main elements including photosensitizer, harmless visible light, and reactive oxygen species (ROS) [18,19]. Among the factors influencing the efficiency of PDT, the photosensitizer has a key role [20]. Owing to some predominant characteristics of metal phthalocyanine (the second generation of photosensitizers) such as high stability, high optical toxicity, low dark toxicity, strong absorption in phototherapeutic window (a wavelength range of 600 to 900 nm), high ROS production, and selective absorption by malignant tissues, zinc phthalocyanine is considered among the most promising photosensitizers used in PDT [21–24].

The photosensitizer can be placed within the organelles such as mitochondria, lysosome, endoplasmic reticulum (ER), Golgi, and the plasma membrane [25]. It has to be noted that the type of cell death after PDT is highly dependent on replacement of photosensitizer inside the cells due to the instability of reactive oxygen species (half-life from 1 to 4 microseconds and the radius migration of < 30 nm [26]) [27]. In addition, the type of cell death also depends on photosensitizer, the

* Corresponding author.
E-mail address: tajalli@tabrizu.ac.ir (H. Tajalli).

http://dx.doi.org/10.1016/j.jphotobiol.2017.09.020
Received 15 June 2017; Received in revised form 3 September 2017; Accepted 21 September 2017
Available online 22 September 2017
1011-1344/

total delivered energy, the type of cells, and the applied protocol [28–31].

Mechanisms of tumor destruction in the PDT include direct effects (direct damage to tumor cells via apoptosis/necrosis) and indirect effects (damage to the blood vessels supplying the tumor tissue and host immune system activation against the tumor) [32]. PDT can induce different cell death mechanisms such as apoptosis/necrosis [33]. Apoptotic cell death mechanism is an ATP-dependent process [34], activated by external stimuli such as TNF-α, Fas, and TRAIL or through internal stimuli and mitochondrial signaling pathway [35,36].

In terms of morphology, features of apoptosis include mitochondrial membrane permeability, condensation chromatin, fragmentation of DNA, cell shrinkage, bubbled plasma membrane, the formation of apoptotic bodies and exposed phosphatidylserine to the outer layer of the plasma membrane. At the biochemical level, apoptosis is a caspase-dependent phenomenon and various morphological changes in cells are caused by caspases activity [37,38]. However, according to available studies, caspase-independent apoptosis is also possible to occur. The main mediator in the caspase-independent apoptosis is an apoptosis-inducing factor (AIF) released from mitochondria. In caspase-independent apoptosis, morphology of the nucleus such as a part of chromatin in nucleus is condensation without DNA fragmentation. This feature is distinct from apoptosis, while many other features of apoptosis have maintained [39–42].

Cellular and molecular mechanisms of apoptosis induced by PDT are highly complex and not fully known. Therefore, characterizing these mechanisms leads to design better PDT protocols that widely enhance its effectiveness [25,43].

In this study, the effectiveness of PDT on SK-MEL-3 cell line using different concentrations of Zinc-Phthalocyanine as a photosensitizer and a diode laser with a wavelength of 675 nm is evaluated. The purpose of this study is to compare the effects of different laser doses on the type of induced cell death and, finally, the analysis of cellular and molecular mechanisms involved in this process.

2. Materials and Methods

2.1. Cell Culture

The SK-MEL-3 cell line was purchased from the Pasteur Institute (Tehran, Iran). Firstly, the cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS, Gibco, USA) and antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin) (Gibco, USA). Consequently, cells were incubated at 37 °C in 5% CO_2 and 95% humidity until they reached a 70% confluency. The passage number of cells were 3 for all experiments.

2.2. Photosensitizer

Zinc phthalocyanine (ZnPc) was purchased from Sigma-Aldrich (USA). Since ZnPc is strongly hydrophobic, dimethylsulfoxide (DMSO) was used to make ZnPc dissolve in the culture medium [44]. The stock solution of ZnPc (5 μg/mL) was prepared using DMSO and RPMI1640 (Gibco, USA) and then sonicated in a sonicator (bath type, Elma transonic T420, Germany). The total concentration of DMSO in the stock solution was reached 2% (v/v) in RPMI1640. In other words, ZnPc was firstly dissolved in DMSO by sonication, and then gradually added to RPMI-1640 medium until the final concentration of ZnPc reached 5 μg/mL including 2% (v/v) DMSO. All the concentrations (0.00001–5 μg/mL) tried in the experiments were diluted using RPMI1640. It is worth mentioning that ZnPc has a weak absorption peak at 345 nm and a strong one at 675 nm [1,45].

2.3. Light Source

As stated above, the strong absorption peak of ZnPc is indexed at 675 nm; therefore, to match the wavelength light source with the maximum absorption of ZnPc, a continuous wave diode laser (Shenzhen Taiyong Technology, China) with a wavelength of 675 nm and 80 mW output power at different times including 20, 40, and 60 s (i.e., 8, 16, and 24 J/cm^2 laser doses, respectively) was utilized.

2.4. PDT Treatment

For in vitro PDT, after reaching almost a 70% confluency of cells in the flask, they were centrifuged, counted, and seeded in 96-well plates with the number of 10,000 cells per well. Then, they were incubated overnight (at 37 °C, 95% humidity, and 5% CO_2). The examined cells in this study were categorized into four different groups. The first group, specified as the control group, received neither ZnPc nor laser. The second group was treated only with different concentrations of ZnPc without laser irradiation. The third group, defined as laser control group, was exposed to different doses of the laser (8, 16, and 24 J/cm^2) in the absence of ZnPc. The final group received both ZnPc and laser exposure.

To carry out the examinations, firstly, the second and fourth groups were incubated with different concentrations of ZnPc in the total dark condition. After 24 h, all the wells were rinsed with PBS. Next, the cells in the groups three and four were exposed to the laser with three different doses in a dark room and eventually incubated for 24 h at 37 °C, 95% humidity, and 5% CO_2.

2.5. MTT Assay

According to our previously reported article [1] and with slight changes, cytotoxic effects of PDT treatment with ZnPc were investigated using methylthiazole tetrazolium kit (MTT assay, Sigma). In brief, 24 h after laser irradiation, 50 μL of MTT solution (2 mg/mL in PBS) was added to each well and incubated at 37 °C for 4 h. This process led to metabolize of the MTT to formazan crystals by the succinate-tetrazolium reductase system active only in viable cells. To dissolve the insoluble formazan crystals, 200 μL DMSO was added to each well and incubated for 20 min. Eventually, optical density (OD) of each well was measured at the wavelength of 570 nm using an ELISA reader (Sunrise ELISA Plate Reader, Tecan, Salzberg, Austria). All the tests were done in dark condition and repeated three times. To obtain the percentage of viable cells in each well, the following formula was used.

$$\text{Cell viability (\%)} = (\text{OD of sample}/\text{OD of control}) \times 100$$

2.6. DAPI

The changes in the cell nucleus morphology characteristic of apoptosis were examined by citation 5 system (Biotek, USA) of DAPI-stained cells. The SK-MEL-3 cells were seeded 5000 cells per well in a 96 well culture plate. After 24 h, PDT treatment with ZnPc was performed on three previously introduced groups. The cells were fixed with 4% paraformaldehyde for 10 min. The plate was washed twice for 3 min with PBS. Next, the cells were permeable by 0.1% Triton-X-100 for 10 min. Then, after washing three times, the cells were stained with 0.15% DAPI in PBS for 10 min at room temperature (18–20 °C) in the dark. Triplicate random fields were chosen for the experiment. Subsequently, a ratio of the cells showed some evidence of apoptosis.

2.7. Detection of Apoptosis/Necrosis by Annexin-V-FLUOS Staining

The apoptosis/necrotic percentages of cells after PDT treatment in three obtained IC_{50}s were examined using Annexin-V-FLUOS staining Kit (Roche, Germany). Shortly, 24 h after the treatment of SK-MEL-3 cells with PDT, cells were washed twice with PBS and centrifuged. Afterward, the suspended cells were stained with 1 μL Annexin and 1 μL PI and incubated again in the dark for more 15 min. At last, cell

apoptosis/necrosis was detected and analyzed using flow cytometry (MacsQuant Analyser, Miltenyi Biotech, Germany).

2.8. Real-time Quantitative PCR

To make the results more convincing, Real-time tests were performed by taking the following steps: First, total-RNA extraction of the PDT pre-treated cells (at three obtained IC_{50}s) was done using GeneAll (Korea) kit according to the manufacturer's protocol. Second, mRNA was converted to cDNA by PrimeScript Reverse Transcriptase and oligo dT primer using cDNA synthesis kit (TAKARA, Japan) according to the manufacturer's protocol. The QRT-PCR method was applied to measure the mRNA expression levels of caspase-9, -8, -3 and Bcl-2 using SYBR Premix (Ampliqon, Denmark) and Light Cycler system (Roche, Roche Diagnostics). The reaction system of PCR includes 5 μL SYBR Green reagent, 0.25 μL of each 4 pmol primers, 0.5 μL cDNA template, and 4.25 μL nuclease-free distilled water. Prior to use, all pair primer sequences used in this study were blasted according to primer-blast software available on NCBI website (http://www.nchi.nlm.nih.gov). The primer sequences used for Bcl-2, caspase -8, -9, -3, and GAPDH genes are shown in Table 1. GAPDH was used as the reference gene. The relative levels of gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method. All QRT-PCR reactions were carried out triplicate.

2.9. Statistical Analysis

Data were analyzed via GraphPad Prism version 3 statistical software. All values are expressed as means ± standard deviation (SD) and all analyses have been repeated at least three times. Student's *t*-test and ANOVA were used to determine the statistical significance of differences between groups. Differences with a p value < 0.05 were considered to be significant.

3. Results and Discussion

According to studies, after PDT, different intracellular signals may be activated, determining the death or survival of cancer cells. As mentioned before, activation of these signals depends on the cell type, genetic, metabolic potentials, used protocol, the total amount of energy delivered to the system, type of PS, and the intracellular localization of PS [28–31]. Nowadays, an effective treatment of cancer not only corresponds to the high death rate of cancer cells but also is pertinent to the death type. The induced apoptosis as a programmed cell death is the core of all research in cancer treatments.

3.1. Cytotoxicity Effects of ZnPc and Light Source on SK-MEL-3 Cell Line

The effects of different concentrations of ZnPc and three doses of the laser on SK-MEL-3 cell line were evaluated. The obtained data (Fig. 1) shows that in the absence of light (1A), ZnPc had no palpable effects on cells. Similarly, laser exposure with no ZnPc (1B) did not leave any cytotoxic effects on SK-MEL-3 cells. The lack of PS toxicity in the dark and the harmlessness of light source are the primary points in PDT [46]. However, the excited ZnPc in the account of laser irradiation led to a concentration-dependent cell death (1C, 1D, 1E). The IC_{50} values of ZnPc for SK-MEL-3 cells in three doses of laser 8, 16, and 24 J/cm^2 were 0.064 ± 0.01, 0.043 ± 0.01, and 0.036 ± 0.01 μg/mL (Table 2), respectively. Based on the obtained IC_{50}s at low concentrations, it is turned out that the ZnPc phototoxic activity was highly dependent on ZnPc concentration. Thus, the obtained cytotoxic and phototoxic results were an evidence to prove the high performance of ZnPc at low concentrations during PDT in the SK-MEL-3 cell line, indicating the great effectiveness of the treatment with minor invasive impacts.

Table 1
Primers sequences.

Genes	Primer sequences	
Bcl-2	Forward	5′ CCTGTGGATGACTGAGTACC 3′
	Reverse	5′ GAGACAGCCAGGAGAAATCA 3′
Caspase-8	Forward	5′ GGTCTGAAGGCTGGTTGTTC 3′
	Reverse	5′ AATCTCAATATTCCCAAGGTTCAAG 3′
Caspase-9	Forward	5′ CCGGAATCCTGCTTGGGTATC 3′
	Reverse	5′ CATCGGTGCATTTGGCATGTA 3′
Caspase-3	Forward	5′ TGTCATCTCGCTCTGGTACG 3′
	Reverse	5′ AAATGACCCCTTCATCACCA 3′
GAPDH	Forward	5′ CCTCGTCCCGTAGACAAAA 3′
	Reverse	5′ AATCTCCACTTTGCCACTG 3′

3.2. Apoptosis/Necrosis of SK-MEL-3 Detection by ZnPc After Irradiation

PDT can lead to induction of apoptosis or necrosis [25,47]. Utilizing low doses of laser and low concentrations of PS in PDT are prominent factors to induce apoptosis in the cells [30,48–50]. Also, a shorter ZnPc incubation time causes a high rate of necrosis [43]. Such a high rate explains why we use the low doses of the laser, low concentrations and long incubation time of ZnPc to have the maximum ratio of apoptosis and minimum necrosis. To understand if apoptosis is the major event after PDT with ZnPc, DAPI staining was performed. The cells with nuclear fragmentation in DAPI staining correspond for apoptosis induced cells (Fig 2).

As shown in Fig. 3, flow cytometry results represented that the untreated control cells, primarily incubated by FITC and PI, were approximately viable and no apoptosis and no necrosis occurred. Meanwhile, after treatment with PDT in three doses four groups including viable cells (FITC and PI negative), necrosis (FITC negative and PI positive), cells undergoing early apoptosis (FITC positive and PI negative), and cells in the late stage of apoptosis (FITC and PI positive) appeared. As a result, we can conclude that PDT with ZnPc can induce a high level of apoptosis in treated cells and keep the ratio of necrosis negligible (< 1%). In other words, the high percentage of apoptotic cells were seen in the stage of early apoptosis 24 h after PDT. Therefore, it might be right if we say that the initial damage site of PDT is not mitochondria. Because if PDT damaged mitochondria, the apoptosis process would have quickly happened by losing the whole of the mitochondrial membrane as soon as the photodynamic stress was imposed. Likely, based on the available studies, the slow process of apoptosis induction after PDT is due to the localization of ZnPc in lysosomes or plasma membrane [51].

3.3. Caspase-8, -9, -3 and Bcl-2 Genes Expression by PDT With ZnPc

Accordingly, in this study, we examined the molecular mechanisms responsible for induction of apoptosis. Interactions of death receptors such as Fas and tumor necrosis factor (TNF) with their ligands on the cell surface led to activation of initiator caspase-8 that consequently contributed to the activation of executioner caspase-3 and triggering the extrinsic pathway apoptosis [52]. The Bcl-2 protein is an anti-apoptotic member of the Bcl-2 family. This protein has an important role in preserving the integrity of mitochondria by blocking the outer mitochondrial membrane channels [25]. In the case of inhibition of Bcl-2 protein and mitochondrial outer membrane permeability, cytochrome C is released and causes the formation of apoptosome complex that leads to the activation of initiator caspase-9, activation of executioner caspase-3, and triggering the intrinsic pathway apoptosis [53].

Hence, in this study, the changes in the expression of Bcl-2 encoding gene were examined, followed by investigating the relationship between induced apoptosis and the activity of caspase-8 and -9 and their role in activation of caspase-3. In this regard, the changes in the expression of this gene were assessed using QRT-PCR 24 h after PDT. The analysis of QRT-PCR results is illustrated in Fig. 4.

While plasma membrane was the initial damage site of PDT, the

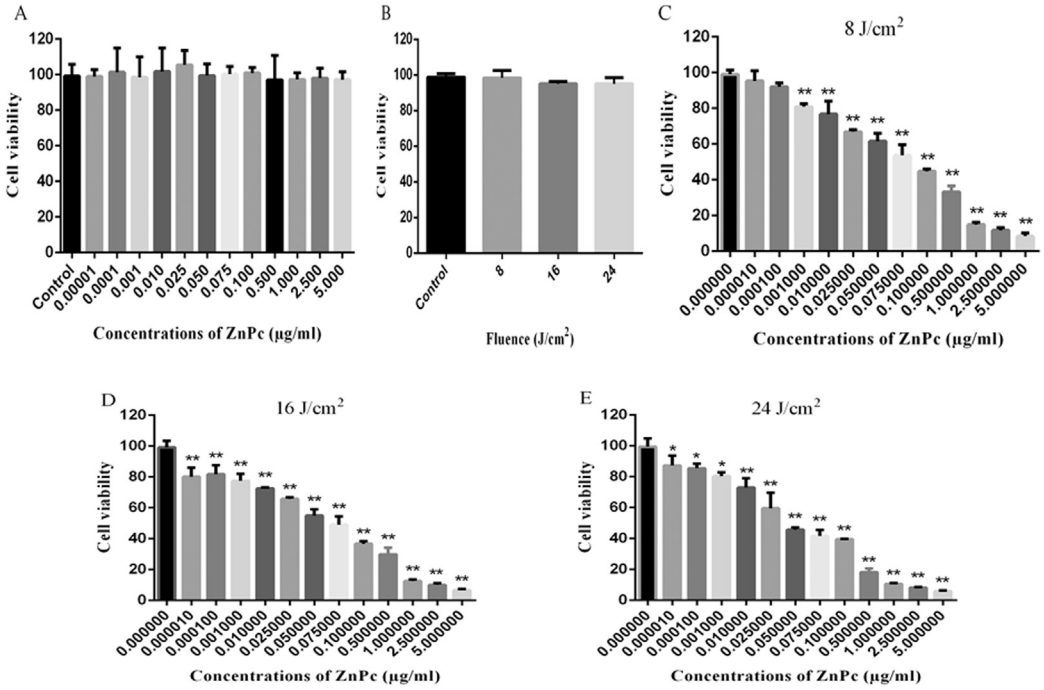

Fig. 1. Cytotoxic effects of ZnPc (with or without irradiation) and light source on SK-MEL-3 cell line: (A) The cell viability in presence of 0, 0.00001, 0.0001, 0.001, 0.01, 0.025, 0.05, 0.075, 0.1, 0.5, 1, 2.5, and 5 µg/mL of ZnPc without irradiation for SK-MEL-3 cells; (B) The effect of irradiation of 8, 16 and 24 J/cm^2 laser dose on SK-MEL-3 cells without ZnPc. The viability of SK-MEL-3 cells after incubation with different concentration of ZnPc (0 to 5 µg/mL) for 24 h and exposing to laser at (C) 8, (D) 16, and (E) 24 J/cm^2. The results are expressed as mean ± SD (n = 3); $^*p < 0.02$, $^{**}p < 0.0001$ versus control.

Table 2
IC_{50} values in different doses of laser.

Doses of laser (J/cm^2)	IC_{50} values (µg/mL)
8 J/cm^2	0.064 ± 0.01
16 J/cm^2	0.043 ± 0.01
24 J/cm^2	0.036 ± 0.01

apoptosis probably is associated with activation of caspase-8 [54,55]. Subsequently, the activation of caspase-8 led to cytochrome C release, caspase-3 activation, and apoptosis induction [56]. It was also reported that if ZnPc is mainly localized in the lysosome, the probability of activation of caspase-8 through death receptors was not likely to happen [52]. Accordingly, in this study, the gene expression changes of caspase-8 were studied. Upon to the results of QRT-PCR, it is obvious that the gene expression of caspase-8 in three investigated doses reduced in comparison to control group; thus, the induced apoptosis does not occur

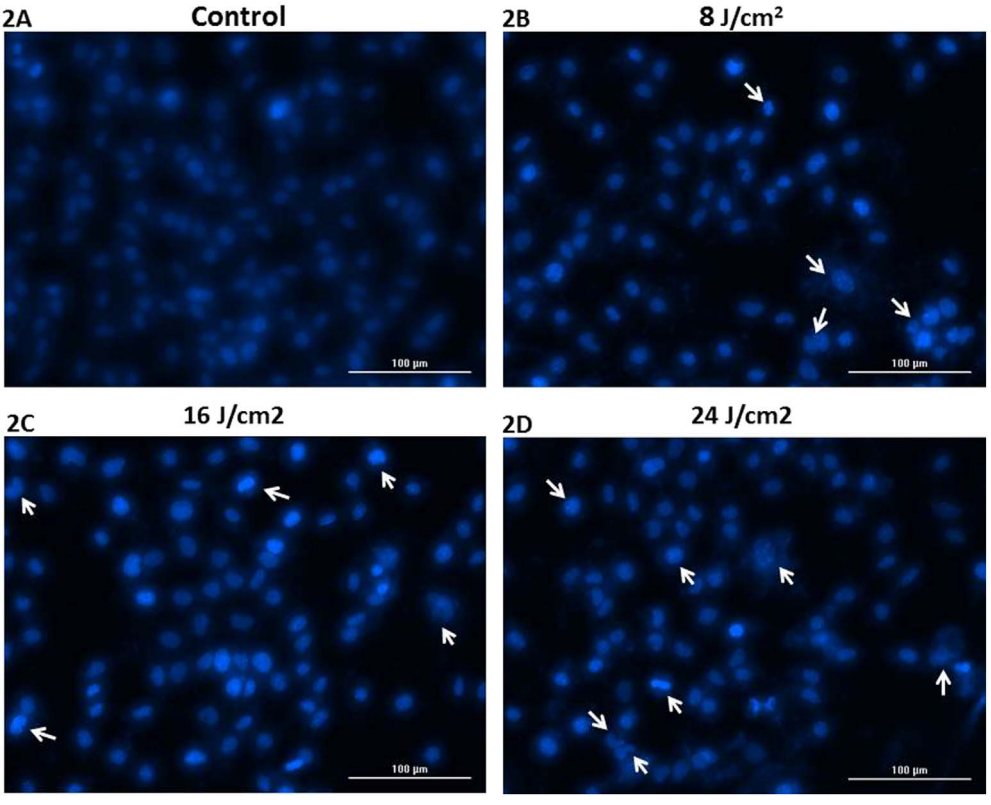

Fig. 2. The apoptotic cell death in skin cancer cells as a result of exposing ZnPc to the laser. The cells with nuclear fragmentation in DAPI staining (A) control (untreated cells), (B) 8, (C) 16, and (D) 24 J/cm^2.

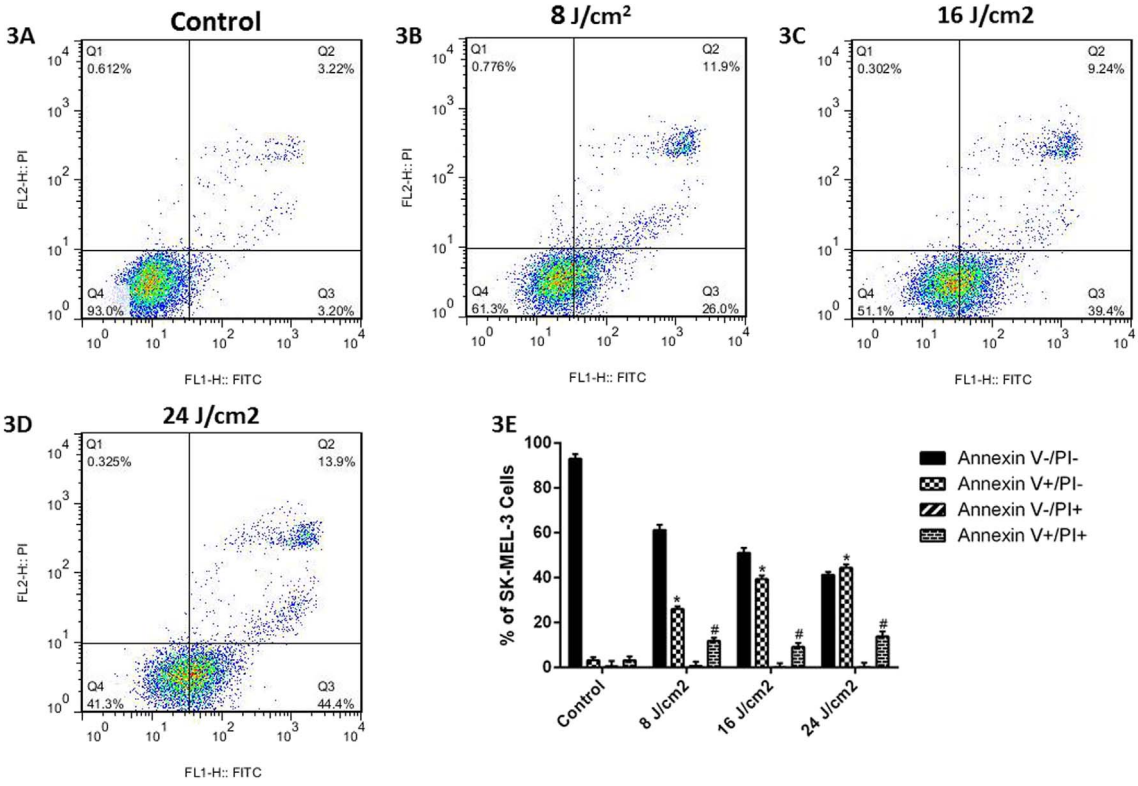

Fig. 3. Apoptosis and necrosis effects on SK-MEL-3 induced by PDT: (A) control (untreated cells); cells were incubated with (B) 0.064, (C) 0.043, and (D) 0.036 μg/mL ZnPc for 24 h and exposed to light dose (B) 8, (C) 16, and (D) 24 J/cm^2, respectively. (E) induction of apoptosis or necrosis by ZnPc-PDT by flow cytometry Annexin V/PI analyzed 24 h post-PDT. After treatment with PDT in three doses, there were four groups including viable cells (Annexin V − /PI −), necrosis cells (Annexin V − /PI +), early apoptosis (Annexin V + /PI −), and late apoptosis (Annexin V + and PI +) compared with the untreated controls.

through the extrinsic pathway. Also, the slow process of apoptosis induction might be because of ZnPc localization in the lysosome, not plasma membrane. If the PS mainly be located in the lysosome, the caspase-dependent or caspase-independent apoptosis after PDT would be observed [34,58] and the apoptosis pathway begins with a slow rate through the mitochondrial pathway. So, the photodamage of lysosome causes lysosomal membrane to be permeable and to leakage of lysosomal enzymes including cathepsins into the cytoplasm. Cathepsin induces Bid activation and thereby the death signal is transmitted from the lysosome to mitochondria, leading to apoptosis [51,57]. Bid protein inhibits Bcl-2 proteins. Hence, as results show, the expression of Bcl-2 in all three doses reduced compared to the control group, which was an

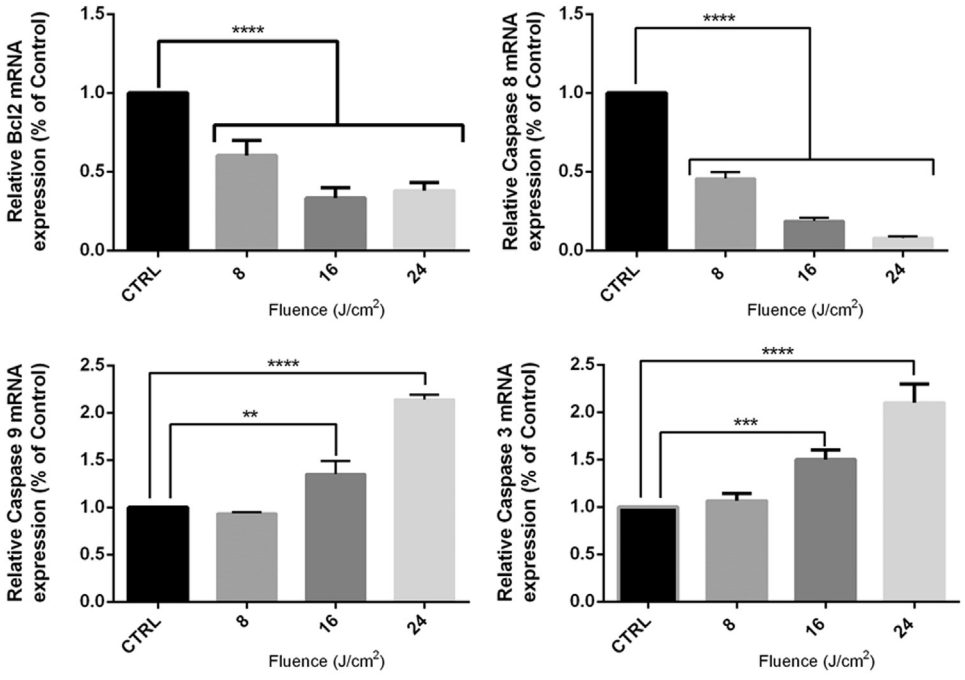

Fig. 4. The effect of ZnPc-PDT on Bcl-2 and caspase-8, -9, -3 gene expression. SK-MEL-3 cells were incubated 24 h with 0.064, 0.043, and 0.036 μg/mL of ZnPc and exposed to light dose of 8, 16, and 24 J/cm^2, respectively. The mRNA Relative expression of (A) Bcl-2, (B) Caspase-8, (C) Caspase-9, and (D) Caspase-3 measured by QRT-PCR using $2^{(-\Delta\Delta Ct)}$ method. The results are expressed as mean ± SD (n = 3). $^{**}p < 0.001$, $^{***}p = 0.0001$ and $^{****}p < 0.0001$ versus control.

indication of anti-apoptotic agent inhibition. Consequently, the loss of mitochondrial membrane integrity occurs [52].

Following by examining and comparing the changes of caspase-9 and -3 gene expression at three doses, it was found that apoptosis was induced in two doses (16, 24 J/cm^2) by activation of caspase-9 and subsequently caspase-3. But, in the dose of 8 J/cm^2 none of the initiator and executioner caspases were activated; thus, in this dose of PDT, the apoptosis was probably caspase-independent. In addition to the classic pathway of caspase-dependent apoptosis, in some cases, damage to the lysosome can lead to caspase-independent apoptosis. In caspase-independent apoptosis, the lysosomal enzymes influenced the Bid protein and ultimately transmitted the death signal to mitochondria. One of the contents released from the mitochondria was AIF that, contributing calpain-like protease, induced the caspase-independent apoptosis [34]. The importance of caspase-independent apoptosis is due to lysosome damage, particularly in cancer cells. Although some cancer cells may have blocked the classic apoptosis pathways (due to genes mutation involved in apoptosis), apoptosis can be still induced in these cells by damage to the lysosome.

At the first glimpse, it may seem that the impact of utilizing different doses of laser in the present study on the amount and type of cell death is imperceptible. However, examinations and thorough investigations in comparing the activated molecular pathways in three different laser doses unveiled the considerable role of different laser doses in activation of different molecular pathways in treated cells. On this basis, we can understand the complexity of PDT parameters' effects on the intracellular signals. However, further studies and investigations are highly recommended in this field.

4. Conclusion

In the present study, apoptosis inducer molecular pathways during PDT were examined with three different laser doses and ZnPc different concentrations on the SK-MEL-3 cancer cells. According to the obtained results, the started slow apoptosis pathway was due to the localization of ZnPc, mainly, within the lysosome. As a result of ZnPc positioning in the lysosome, caspase-dependent or caspase-independent apoptosis were induced, within which the high doses of laser caused caspase-dependent apoptosis, while low doses of laser caused the caspase-independent apoptosis. Therefore, the applied laser doses had a predominant role in determining the both type of apoptosis molecular pathways. However, the molecular mechanisms and other apoptosis inducer factors during PDT are very intricate and not fully known yet; and so more studies are needed in this regard.

References

[1] F. Navaeipour, et al., Effects of continuous wave and fractionated diode laser on human fibroblast cancer and dermal normal cells by zinc phthalocyanine in photodynamic therapy: a comparative study, J. Photochem. Photobiol. B Biol. 161 (2016) 456–462.

[2] A.M. Garcia, et al., Photodynamic performance of zinc phthalocyanine in HeLa cells: a comparison between DPCC liposomes and BSA as delivery systems, J. Photochem. Photobiol. B Biol. 163 (2016) 385–390.

[3] A. Gupta, et al., Shining light on nanotechnology to help repair and regeneration, Biotechnol. Adv. 31 (5) (2013) 607–631.

[4] L. Corti, et al., Outcome of patients receiving photodynamic therapy for early esophageal cancer, Int. J. Radiat. Oncol. Biol. Phys. 47 (2) (2000) 419–424.

[5] L. Howe, J. Zhang, The effect of biological substrates on the ultrafast excited-state dynamics of zinc phthalocyanine tetrasulfonate in solution, Photochem. Photobiol. 67 (1) (1998) 90–96.

[6] C.-Y. Wang, et al., Photosensitization of phycocyanin extracted from Microcystis in human hepatocellular carcinoma cells: implication of mitochondria-dependent apoptosis, J. Photochem. Photobiol. B Biol. 117 (2012) 70–79.

[7] T.A. Debele, S. Peng, H.-C. Tsai, Drug carrier for photodynamic cancer therapy, Int. J. Mol. Sci. 16 (9) (2015) 22094–22136.

[8] L.-Y. Xue, S.-M. Chiu, N.L. Oleinick, Atg7 deficiency increases resistance of MCF-7 human breast cancer cells to photodynamic therapy, Autophagy 6 (2) (2010) 248–255.

[9] D. Bechet, et al., Photodynamic therapy of malignant brain tumours: a complementary approach to conventional therapies, Cancer Treat. Rev. 40 (2) (2014) 229–241.

[10] J. Usuda, et al., Photodynamic therapy (PDT) for lung cancers, J. Thorac. Oncol. 1 (5) (2006) 489–493.

[11] S. Pushpan, et al., Porphyrins in photodynamic therapy-a search for ideal photosensitizers, Curr. Med. Chem. Anticancer Agents 2 (2) (2002) 187–207.

[12] H. Kolarova, et al., In vitro photodynamic therapy on melanoma cell lines with phthalocyanine, Toxicol. in Vitro 21 (2) (2007) 249–253.

[13] C. Fidanzi-Dugas, et al., Analysis of the in vitro and in vivo effects of photodynamic therapy on prostate cancer by using new photosensitizers, protoporphyrin IX-polyamine derivatives, Biochim. Biophys. Acta Gen. Subj. (2016).

[14] S. Haywood-Small, et al., Phthalocyanine-mediated photodynamic therapy induces cell death and a G 0/G 1 cell cycle arrest in cervical cancer cells, Biochem. Biophys. Res. Commun. 339 (2) (2006) 569–576.

[15] H.H. Lee, M.-G. Choi, T. Hasan, Application of photodynamic therapy in gastrointestinal disorders: an outdated or re-emerging technique? Korean J. Intern. Med. 32 (1) (2017) 1.

[16] E. Spyratou, et al., Biophotonic techniques for manipulation and characterization of drug delivery nanosystems in cancer therapy, Cancer Lett. 327 (1) (2012) 111–122.

[17] H.I. Pass, Photodynamic therapy in oncology: mechanisms and clinical use, J. Natl. Cancer Inst. 85 (6) (1993) 443–456.

[18] M. Weller, et al., European Association for Neuro-Oncology (EANO) Task Force on Malignant Glioma. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma, Lancet Oncol. 15 (9) (2014) e395–e403.

[19] O.O. Kanu, et al., Glioblastoma multiforme oncogenomics and signaling pathways, Clin. Med. Oncol. 3 (2009) 39.

[20] J. Shao, et al., Intracellular distribution and mechanisms of actions of photosensitizer Zinc (II)-phthalocyanine solubilized in Cremophor EL against human hepatocellular carcinoma HepG2 cells, Cancer Lett. 330 (1) (2013) 49–56.

[21] Y. Zorlu, et al., Monoglycoconjugated water-soluble phthalocyanines. Design and synthesis of potential selectively targeting PDT photosensitisers, Tetrahedron Lett. 51 (50) (2010) 6615–6618.

[22] W.M. Sharman, S.V. Kudrevich, J.E. van Lier, Novel water-soluble phthalocyanines substituted with phosphonate moieties on the benzo rings, Tetrahedron Lett. 37 (33) (1996) 5831–5834.

[23] K. Oda, S.-i. Ogura, I. Okura, Preparation of a water-soluble fluorinated zinc phthalocyanine and its effect for photodynamic therapy, J. Photochem. Photobiol. B Biol. 59 (1) (2000) 20–25.

[24] C.M. Allen, W.M. Sharman, J.E. Van Lier, Current status of phthalocyanines in the photodynamic therapy of cancer, J. Porphyrins Phthalocyanines 5 (2) (2001) 161–169.

[25] P. Mroz, et al., Cell death pathways in photodynamic therapy of cancer, Cancer 3 (2) (2011) 2516–2539.

[26] S. Bhattacharjee, The language of reactive oxygen species signaling in plants, J. Bot. 2012 (2012).

[27] G. Nam, et al., Cell death mechanistic study of photodynamic therapy against breast cancer cells utilizing liposomal delivery of 5, 10, 15, 20-tetrakis (benzo [b] thiophene) porphyrin, J. Photochem. Photobiol. B Biol. 166 (2017) 116–125.

[28] J.O. Yoo, et al., Differential cytotoxic responses to low-and high-dose photodynamic therapy in human gastric and bladder cancer cells, J. Cell. Biochem. 112 (10) (2011) 3061–3071.

[29] J. Piette, et al., Cell death and growth arrest in response to photodynamic therapy with membrane-bound photosensitizers, Biochem. Pharmacol. 66 (8) (2003) 1651–1659.

[30] C. Fabris, et al., Photosensitization with zinc (II) phthalocyanine as a switch in the decision between apoptosis and necrosis, Cancer Res. 61 (20) (2001) 7495–7500.

[31] A.P. Castano, P. Mroz, M.R. Hamblin, Photodynamic therapy and anti-tumour immunity, Nat. Rev. Cancer 6 (7) (2006) 535–545.

[32] W. Li, Q. Ma, E. Wu, Perspectives on the role of photodynamic therapy in the treatment of pancreatic cancer, Int. J. Photoenergy 2012 (2011).

[33] P.E. Feuser, et al., Synthesis of ZnPc loaded poly (methyl methacrylate) nanoparticles via miniemulsion polymerization for photodynamic therapy in leukemic cells, Mater. Sci. Eng. C 60 (2016) 458–466.

[34] N.B.R. Vittar, et al., Caspase-independent apoptosis, in human MCF-7c3 breast cancer cells, following photodynamic therapy, with a novel water-soluble phthalocyanine, Int. J. Biochem. Cell Biol. 42 (7) (2010) 1123–1131.

[35] G. Kroemer, L. Galluzzi, C. Brenner, Mitochondrial membrane permeabilization in cell death, Physiol. Rev. 87 (1) (2007) 99–163.

[36] J.M. Adams, Ways of dying: multiple pathways to apoptosis, Genes Dev. 17 (20) (2003) 2481–2495.

[37] A. Lüthi, S. Martin, The CASBAH: a searchable database of caspase substrates, Cell Death Differ. 14 (4) (2007) 641–650.

[38] D.R. Green, Apoptotic pathways: ten minutes to dead, Cell 121 (5) (2005) 671–674.

[39] I.E. Furre, et al., Targeting PBR by hexaminolevulinate-mediated photodynamic therapy induces apoptosis through translocation of apoptosis-inducing factor in human leukemia cells, Cancer Res. 65 (23) (2005) 11051–11060.

[40] I.E. Furre, et al., Involvement of both caspase-dependent and-independent pathways in apoptotic induction by hexaminolevulinate-mediated photodynamic therapy in human lymphoma cells, Apoptosis 11 (11) (2006) 2031–2042.

[41] L.E. Bröker, F.A. Kruyt, G. Giaccone, Cell death independent of caspases: a review, Clin. Cancer Res. 11 (9) (2005) 3155–3162.

[42] R.D. Almeida, et al., Intracellular signaling mechanisms in photodynamic therapy, Biochim. Biophys. Acta 1704 (2) (2004) 59–86.

[43] J. Soriano, et al., Regulated necrosis in HeLa cells induced by ZnPc photodynamic treatment: a new nuclear morphology, Int. J. Mol. Sci. 15 (12) (2014) 22772–22785.

[44] N. Nombona, et al., Synthesis of phthalocyanine conjugates with gold nanoparticles

and liposomes for photodynamic therapy, J. Photochem. Photobiol. B Biol. 107 (2012) 35–44.
[45] W. Lü, et al., Synthesis and photoactivity of pH-responsive amphiphilic block polymer photosensitizer bonded zinc phthalocyanine, SCIENCE CHINA Chem. 55 (6) (2012) 1108–1114.
[46] M.P. Siqueira-Moura, et al., Development, characterization, and photocytotoxicity assessment on human melanoma of chloroaluminum phthalocyanine nanocapsules, Mater. Sci. Eng. C 33 (3) (2013) 1744–1752.
[47] D.S. Maranho, et al., Photoinduced nitric oxide and singlet oxygen release from ZnPC liposome vehicle associated with the nitrosyl ruthenium complex: synergistic effects in photodynamic therapy application, Photochem. Photobiol. 85 (3) (2009) 705–713.
[48] J. Shao, et al., Inhibition of human hepatocellular carcinoma HepG2 by phthalocyanine photosensitiser PHOTOCYANINE: ROS production, apoptosis, cell cycle arrest, Eur. J. Cancer 48 (13) (2012) 2086–2096.
[49] N.L. Oleinick, R.L. Morris, I. Belichenko, The role of apoptosis in response to photodynamic therapy: what, where, why, and how, Photochem. Photobiol. Sci. 1 (1) (2002) 1–21.
[50] D.E. Dolmans, D. Fukumura, R.K. Jain, Photodynamic therapy for cancer, Nat. Rev. Cancer 3 (5) (2003) 380–387.
[51] V. Rapozzi, G. Jori, Resistance to Photodynamic Therapy in Cancer, vol. 5, Springer, 2014.
[52] J. Marino, et al., Lysosomal and mitochondrial permeabilization mediates zinc (II) cationic phthalocyanine phototoxicity, Int. J. Biochem. Cell Biol. 45 (11) (2013) 2553–2562.
[53] V. Nikoletopoulou, et al., Crosstalk between apoptosis, necrosis and autophagy, Biochim. Biophys. Acta 1833 (12) (2013) 3448–3459.
[54] S. Zhuang, M.C. Lynch, I.E. Kochevar, Caspase-8 mediates caspase-3 activation and cytochrome c release during singlet oxygen-induced apoptosis of HL-60 cells, Exp. Cell Res. 250 (1) (1999) 203–212.
[55] A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation, Science 281 (5381) (1998) 1305.
[56] M.L. Agarwal, et al., Phospholipase activation triggers apoptosis in photosensitized mouse lymphoma cells, Cancer Res. 53 (24) (1993) 5897–5902.
[57] K. Berg, J. Moan, Lysosomes as photochemical targets, Int. J. Cancer 59 (6) (1994) 814–822.
[58] J. Reiners Jr.et al., Release of cytochrome c and activation of pro-caspase-9 following lysosomal photodamage involves Bid cleavage, Cell Death Differ. 9 (9) (2002) 934.